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STUDIES ON THE VIRUS OF HOG CHOLERA.*†

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INTRODUCTION.

The terms "filterable virus" and "ultramicroscopic organisms" are frequently used in the descriptions of some 30 important infectious diseases of man and animals. These terms constitute a convenient form of expression, as in certain cases it would otherwise be necessary to state "specific cause unknown."

Among the important diseases which fall into this class, as enumerated and briefly described by Wolbach,[1] are yellow fever, molluscum contagiosum, dengue fever, verruca vulgaris, trachoma, sand-fly or three-day fever, acute anterior poliomyelitis, measles, typhus fever, and scarlet fever, which affect man only; rabies, foot and mouth disease, variola, and vaccinia affect both man and animals; whereas hog cholera, fowl pest, cattle plague, sheep pox, African horse sickness, swamp fever of horses, and Rous's chicken sarcoma are among those affecting animals only. Canine distemper, which Wolbach mentions as being produced by a filterable virus, has been demonstrated by Ferry[2] to be due to a bacillus termed by him *B. bronchisepticus*.

Since the recognition of the filterability of hog cholera virus by De Schweinitz and Dorset, 1903–1905, very little progress has been made in elucidating the nature of the specific etiological factor involved.

Dinwiddie,[3] from a series of carefully planned centrifugation experiments, concludes that the infectious agent involved in hog cholera is more or less closely associated with the red blood corpuscles. Aside from the report of Dinwiddie, the knowledge

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[1] *Boston Med. and Surg. Jour.*, 1912, 167, p. 419.

[2] *Jour. Infect. Dis.*, 1911, 8, p. 399.

[3] *Bull.*, Arkansas Exp. Sta., 1912, No. 111.

accumulated in regard to this subject is relatively meager and scattered.

The filterability of a given virus does not preclude the possibility of the presence in that virus of relatively large microorganisms at certain periods, according to the stage of development in their life cycle. Borrel[1] demonstrated a protozoan, *Micromonas mesnili*, which, during certain stages of its development, would pass through the Berkefeld and Chamberland filters.

Loeffler also has shown that a flagellate belonging to the genus Bodo would pass through Berkefeld filters, which were capable of preventing the passage of *B. prodigiosus*, even after an hour's filtration. Thus, filterability cannot be taken as an indication of the size of a given organism, because the passage of organisms through filters depends also on their plasticity and their ability of accommodation to the pores of the filter. In this, the smaller forms of motile parasites differ from bacteria of relatively the same dimensions. It therefore seems possible that some of the filterable viruses causing disease may have a stage in the cycle of their development which is visible under the microscope.

Betegh[2] of Hungary has succeeded in retaining hog cholera virus by means of the Bechhold ultra-filter. His experiments consisted in the filtration of two different strains of virus and the injection of each of the filtrates into three susceptible pigs. The six pigs remained well and the conclusion was that the virus had remained in the ultra-filter.

In 1910,[3] a comparative histological study of the blood of normal hogs and cholera-infected animals was conducted. In this work, the blood of hogs suffering from cholera, in comparison with normal blood, was studied in a routine way on the hemocytometer and with the ordinary blood stains, but at the same time much care was exercised in attempting to find any differences which might indicate the probable nature of the causative factor. Aside from the data obtained relative to the ordinary blood examinations, nothing of note was observed in the specimens of hog cholera blood.

A few months ago a comparative study of normal hog blood and blood from animals suffering from hog cholera was undertaken, in order to determine whether any differences could be detected by means of the dark field method of illumination. A preliminary report[1] of this work has appeared.

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[1] *Ann. de l'Inst. Pasteur*, 1903, 17, p. 81.

[2] *Ber. thierärztl. Wchnschr.*, 1912, 28, p. 969.

[3] King and Wilson, *Bull.* Kansas Exp. Sta., 1910, No. 171.

The fresh blood of normal and diseased hogs was collected aseptically in sterile sodium citrate solution and kept in the incubator until the examination was completed. The blood was obtained from the caudal artery after a small portion had been clipped off with a sterile instrument. In this way, bacterial contamination was reduced to the minimum.

In the blood of both normal hogs and animals suffering from hog cholera, as in the blood of any animal, many peculiar structures are observable. The blood of normal hogs thus examined on the dark field may show, in addition to the normal structures, a few granular bodies, sometimes a few bacterial cells from possible extraneous contamination and filaments. The latter may assume the form of "dumb bells," "chains," "droplets" or flexible filaments, which by an untrained observer might easily be mistaken for spirochetes. An excellent description of these bodies as well as others found in the blood of normal animals is given by Balfour.[2]

Betegh, in examining the hog lymph and serum respectively, from two animals dead from hog cholera, evidently has mistaken some of these filaments, described by Balfour, for bodies of some possible significance. In his very recent article on ultrafiltration experiments, Betegh[3] describes, in a rather confused way, his limited dark field findings as follows:

> "Series I. January 16, 1912. Animal infected and died with hog cholera under natural conditions. Pathological changes: Typical stratification (button ulcers) in cecum and colon; edematous infiltration along the arteria coronaria cordis; infiltration in the right apex of lung; disseminated infiltrated areas in the right lung; parenchymatous degeneration of the kidneys; hemorrhagic inflammation of the lymph glands. Diagnosis: Hog cholera. Microscopic findings: Scattered bipolar and other (colon) bacilli in the organs. In the lung lymph beside these, small slightly stained bodies. In the dark field examination, actively motile, massed bodies from 0.3 to 0.5 microns in size, are visible, which are strongly light-refractive. Many appear to have a small apophysis. Very often dumb-bell like forms are seen, and further, spirochete-like microorganisms, which have at each end a light-refracting round knob. Cultural: On

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[1] King and Baeslack, *Jour. Infect. Dis.*, 1913, 12, p. 39.

[2] *Fourth Report*, Wellcome Tropical Research Laboratories, 1911, p. 109.

[3] *Berl. thierärztl. Wchnschr.*, 1912, 28, p. 972.

alkaline agar these have grown at 37° after 24 hours, many coli, bipolar, and bacterial colonies similar to the *B. suipestifer*."

"Series II. April 16, 1912. Swine sickened and died under natural conditions. In colon a typical button ulcer of the size of a small coin. Several lentil-sized ulcers at the point of anastomosis of the small intestine into the colon. The mesenteric lymph glands swollen and hemorrhagically inflamed. The apex of both lungs infiltrated, atelectasis. The pleura was weak and covered with pseudo-membranes. Surface of incision of the lungs marbled; beside yellow necrotic groups, different stages of hepatization of partial pneumonia were visible. Diagnosis: Hog cholera. Microscopically with diluted carbol fuchsin solution, numerous, short bacteria, rounded off at both ends, occasionally typically bipolar colored, were visible. With the Giemsa staining, in part, the same form. Numerous 0.3 to 0.5 micron sized, ovoid or round forms were seen, which seemed to arise from a chromatin substance. In colored streak preparation from the button ulcers, intra- and extra-cellular typical spirochetes were seen. Similar bodies were abundantly visible with the dark field in the serum. They were actively motile. Between small dumb-bell shaped forms were seen also spirochete forms."

From the above description it is quite evident that Betegh based his observations on the fallacies to which Balfour has so aptly called attention. In the blood of different species of normal animals, filaments may be observed which at times closely simulate spirochetes. Most of these "pseudo-spirochetes," as seen in blood on the dark field, present a knobbed appearance at each pole. After one has become accustomed to the appearance of these filaments, one can readily distinguish the lack of motility, in spite of a flexuous distortion, which is assumed as the filaments are carried by a slight current in the fluid of the preparation. These filaments are also lacking in refractibility and are relatively slender in comparison to the spirochetes recognized in this work.

Bacilli are easily recognized in the dark field by the complete rigidity of the cells and, when flagella are present, by the characteristic tumbling motion.

Results from the dark field examination of specimens of blood from some 50 normal hogs indicate that, as a rule, the blood is relatively free from granules. Under ordinary conditions, and with few exceptions, the blood of normal hogs examined has been designated in our notes as "clean." There have been some few instances in which the blood of hogs unexposed to cholera, and in apparent normal condition, have shown the presence of numerous granules. The presence of these granules in normal blood, in the majority of cases, has been traced to ruptured leukocytes.

In the specimens of blood from all infected hogs, which have been observed by means of the dark field, a relatively large spirochete has been found. It averages from five to seven microns in length and one micron in width. The body of the organism is flexible and round at its ends. It presents no knobbed appearance at its poles. Actively motile, it revolves about its longitudinal axis. Its motility is undulating in character and its spirals are fixed. A few of these organisms have been observed dividing longitudinally. In one permanent microscopical mount, prepared by india ink fixation, one of these organisms apparently shows a polar flagellum. On the dark field this spirochete is readily distinguished from bacteria on account of its lack of rigidity and its characteristic motility, and from "blood filaments" by its greater refractive properties and characteristic morphology.

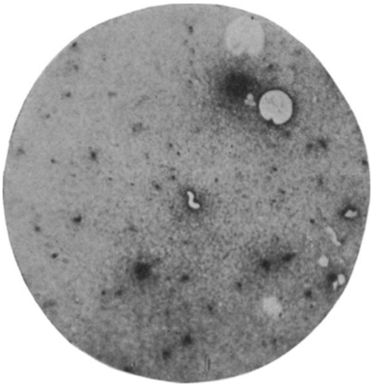

FIG. 1.—Microphotograph of spirochete in blood of Hog 504. India ink preparation.

This spirochete has not been found in large numbers, in any of the blood preparations. However, in nearly every specimen examined, more than one has been observed and in many cases five or six have been found with little difficulty. As a rule the organisms have been found to be more numerous at the height of the disease. The specimens of blood examined have been diluted in the proportion of about one to ten or fifteen with sterile sodium citrate solution, which factor should be considered in contemplating the number observed in a given positive specimen. Moreover, it is suggested that this organism, when observed as a spirochete form, constitutes only one stage of its development.

Certain types of granules appear to be characteristic of blood from cholera hogs. It usually contains many granules, some very fine yet more distinct than blood dust, some larger still, and some very distinct, highly refractive bodies. In many specimens of cholera blood were observed innumerable small granules, which were much more definite and distinct in outline than blood dust, and easily differentiated from the whitish, partially refractive granules from ruptured leukocytes and from the more highly refractive and larger bodies composed of débris, bacteria, and filaments. In this work the presence of these granules appeared to be so characteristic of blood from cholera hogs, that it became an invaluable aid in finding the spirochetes. While these particular bodies may be disintegrated blood elements resulting from disease processes, yet it may properly be suggested that some of them may represent certain stages in the life cycle of the spirochete which has been observed.

The majority of the hogs used in these experiments were inoculated with virus which was diluted 1 to 4 with sterile physiologic salt solution and filtered through Berkefeld filters. In the filtrates only a few granules were visible when studied on the dark field.

In this connection it should be mentioned that the majority of the dark field examinations which are included in this work have been checked by each of us. In addition to the above means of controlling the results, specimens of blood were at times collected by an assistant who designated the specimens by symbols. As the daily examinations often included specimens from both normal and diseased animals, the results were put to a practical test and, in no instance, did the results from the dark field examinations deviate from the clinical conditions of the animals whose blood was examined. In some instances, as will be shown by the following detailed results, the presence of hog cholera infections was practically detected by the dark field examination before it was known that the animals showed any symptoms of the disease.

It is interesting to note that in 1894 Dr. Theobald Smith[1] submitted the following brief report on "Coarse and Fine Spirilla in the Intestine of a Hog":

> "The recently published articles on fine spirilla in the excrement of cholera subjects prompt me to communicate an observation from animal pathology. Early in 1889 I found non-liquefying comma bacilli in small abscesses of the large intestine, in a hog which I have briefly described in this journal. In streak preparations of the same, stained with alkaline methylene blue, I found, besides large quantities of vibrios, also

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[1] *Centralbl. f. Bakt.*, 1894, 16, p. 324.

a great many fine spirilla, of two to three wave-lengths. The wave-lengths of fixed form are about two microns. A preparation, now more than five years old, still shows the tiny spirilla very distinctly. At that time they never appeared again in cultures. Further investigations concerning the presence of this organism in other animals have not been made."

Betegh, in a lecture and demonstration given before the Veterinary Congress at Budapest in May, 1912, called attention to the spirochetes which were demonstrable in the ulcers of animals dead from hog cholera.

Little difficulty has been experienced in finding spirochetes in the intestinal ulcers of hogs dead from cholera. In the present work the ulcers, as found in the cecum of cholera-infected hogs, have been obtained as free from contamination as possible, washed with sterile water, and intermittently scraped with sterile instruments. Portions of the scrapings from the diseased submucosa, after the above treatment, have then been examined on the dark field and in stained preparations, preferably by the Giemsa method. While it thus appears possible to demonstrate spirochetes in hog cholera ulcers with uniformity, yet a large variety of bacterial species of course constitute the flora. With present methods of study therefore, the recognition of these spirochetes in the intestinal lesions cannot be taken as of great significance. Whether or not some of these spirochetes in the intestinal lesions bear any relation to those present in the blood of cholera hogs is, of course, an open question.

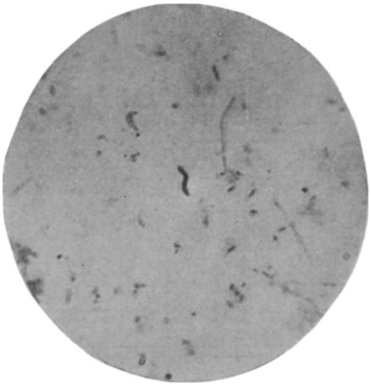


Fig. 2.—Microphotograph, spirochete stained by Giemsa method, from intestinal ulcer of Hog 556.

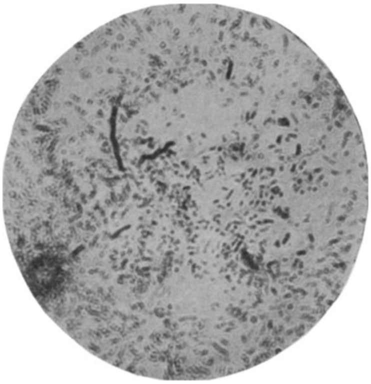


Fig. 3.—Microphotograph of spirochetes in impure culture, from cecal ulcer of Hog 551.

It is possible that the spirochetes found in the blood enter the lymph and blood stream at an early stage of the ulcer formation. This would offer a possible explanation of their comparatively small number as seen on any one blood mount.

The details of the experiments may be better understood by a study of the following temperature charts and laboratory notes. The temperature charts include the time of inoculation, death or release of each animal, and other necessary data including brief notations of clinical conditions and changes observed after death. The results of the dark field examinations are indicated by positive and negative signs placed above the temperature curves on the dates when the observations were made. In some instances the presence of granules or freedom of the blood from granules is indicated by abbreviations, gr. (granules) and c. (clean).

B.A.I. strain of virus.—The history of the strain of virus, designated in these notes as the "B.A.I. strain of virus," was supplied by Dr. Giltner of the Michigan Agricultural College as follows: "This is a representative of the original strain of Bureau of Animal Industry virus secured by Dr. Marshall at the conference at Ames in May, 1908. We have never kept any other strain of virus in the laboratory for any length of time. Practically all of our serum has been produced on the basis of this virus. It would be possible but very difficult to trace the passages through which this virus has gone since we first began to use it."

Beginning with Hog 446 the blood of practically all animals used in these experiments was carefully examined before inoculation in order to control the results.

Hog 446, inoculated with the B.A.I. strain of virus (Hog 444), manifested symptoms of the acute type of hog cholera after an incubation period of seven days. Three positive findings were made in the blood of this animal.

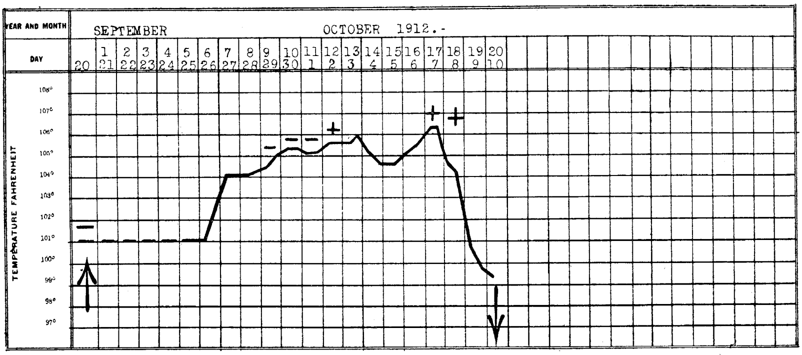
Results from the dark field examinations of the blood of Hogs 453 and 454 afforded most interesting data. Both of these animals recovered after a mild course of the disease following a relatively long period of incubation. As the symptoms in both animals disappeared, dark field examinations failed to reveal the presence of the spirochete and the numerous characteristic granules. These hogs were kept under observation during a period of one month following recovery and were ultimately released as "immune."

Hog 455 was kept in a separate room in the experimental stable from October 26 until November 10 for the purpose of serving as a control on the methods used in isolating the experimental lots of hogs. During a part of this period Hog 455 suffered from a parasitic skin affection, but dark field examinations demonstrated a "clean" blood, free from granules and the spirochete. On November 10 the animal was exposed to the disease and developed symptoms in six days. His blood then showed positive findings and autopsy revealed lesions of cholera.

The charts of Hogs 506 and 507, inoculated with the B.A.I. strain of virus, need no further explanation.

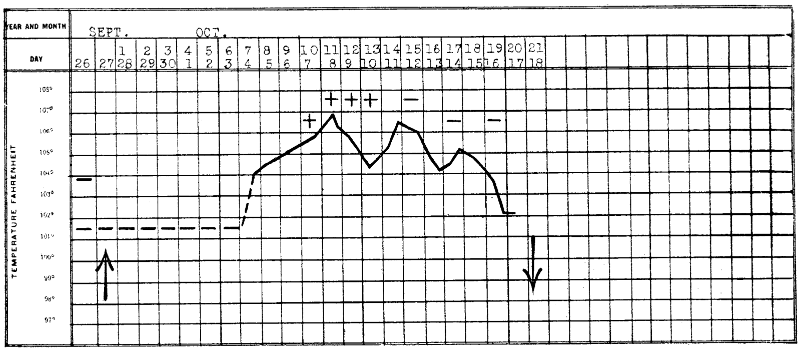
Michigan (Demerick) strain of virus.—On September 24 a farm at Roseville, Mich., was visited for the purpose of examining the herd of hogs. Several sick hogs of this herd, which had been isolated, manifested symptoms similar to those of hog cholera—malaise, anorexia, high fever, and diarrhea. A few purple areas were observed on the abdomen and ears.

HOG NO. 446. B.A.I. STRAIN OF VIRUS.



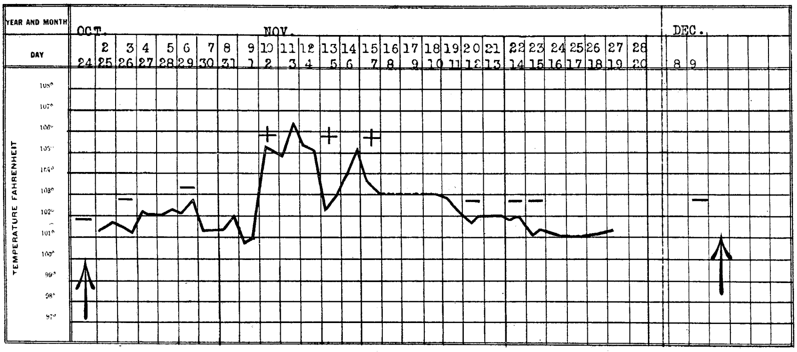
REMARKS: September 20. Animal inoculated with 3 c.c. from 444 (B.A.I.).
September 27. Symptoms appeared.
October 10. Animal moribund, bled and examined.
Skin on ventral surface of body purple in color; hemorrhagic areas in subcutaneous and muscular tissues. Lymphatic glands enlarged and hemorrhagic. Kidneys show a few petechia, ulcers in cecum.

HOG NO. 449. B.A.I. STRAIN OF VIRUS.



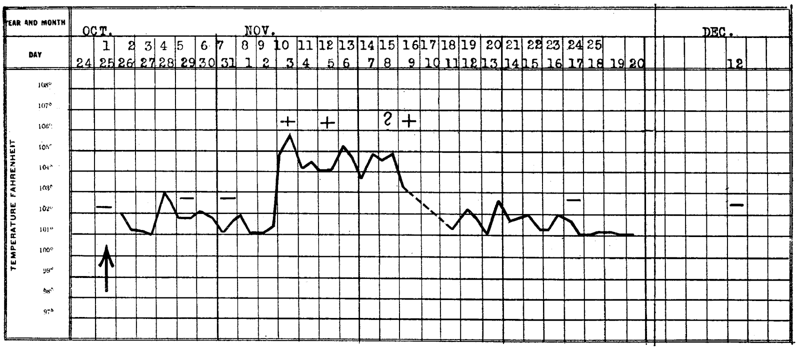
REMARKS: September 27. Inoculated with 4 c.c. B.A.I. virus.
October 5. Symptoms appeared.
October 18. Animal found dead.
Purple hemorrhagic areas extending over greater portion of body externally and into subcutaneous tissue internally. Lymphatic glands enlarged and hemorrhagic. Ulcers in cecum. Kidneys petechiated. Areas of fatty degeneration in liver, right lung, upper lobe solidified.

HOG NO. 453. B.A.I. STRAIN OF VIRUS.



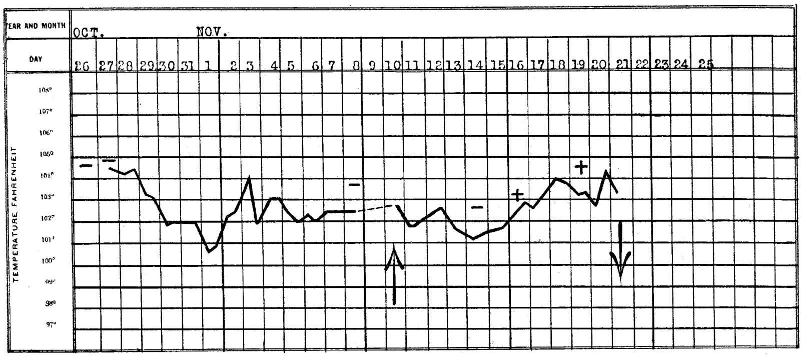
REMARKS: October 24. Inoculated with 4 c.c. B.A.I. (filtered) virus, filtered through Berkefeld.
November 2. Symptoms appeared.
November 5. Blood positive.
November 19. Animal recovered.
December 15. Animal released, immune.

HOG NO. 454. B.A.I. STRAIN OF VIRUS.



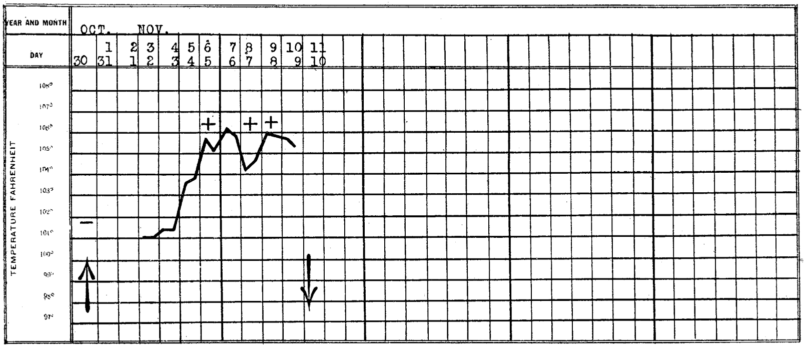
REMARKS: October 25. Inoculated with 4 c.c. B.A.I. virus (unfiltered).
November 2. Symptoms appeared.
November 19. Animal recovered.
December 15. Animal released, immune.

HOG NO. 455. B.A.I. OR MICHIGAN (DEMERICK) STRAIN OF VIRUS.



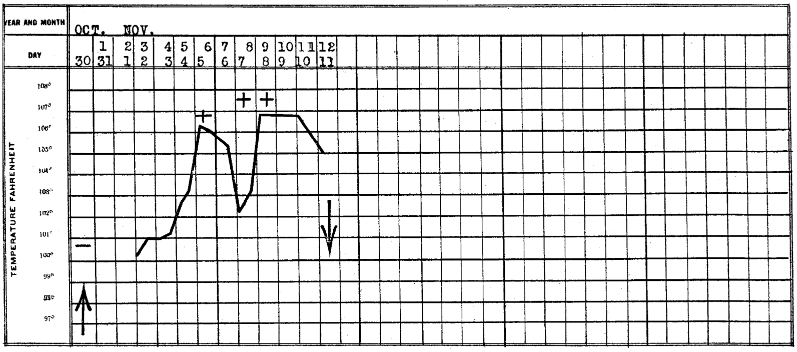
REMARKS: *Control.* October 28. Appearance of "rash" on abdomen and flanks. Animal eats well and shows no symptoms of cholera. Diagnosed as parasitic skin affection.
November 10. Recovering hogs (not dipped) 446, 447, 448 placed in inclosure with 455.
November 16. Animal sick.
November 21. Pig moribund, bled and examined.
Typical lesions in cecum, lymphatic glands, and lungs.

HOG NO. 506. B.A.I. STRAIN OF VIRUS.



REMARKS: October 30. Inoculated with 4 c.c. B.A.I. virus.
November 4. Symptoms appeared.
November 10. Animal found dead and examined.
Cecum, lymphatic glands, spleen, kidneys, and lungs show typical lesions of cholera.

HOG NO. 507. B.A.I. STRAIN OF VIRUS.



REMARKS: October 30. Inoculated with 4 c.c. B.A.I. virus.
November 4. Symptoms appeared.
November 11. Animal found dead and examined.
Lymphatic glands, cecum, lungs, kidneys, and spleen show typical lesions.

With the permission of the owner two of the animals were bled from the carotid artery and careful examinations made. Typical lesions of hog cholera were found in the large intestines, lymphatic glands, lungs, kidneys, and spleen, and a positive diagnosis of the disease in the acute form was made.

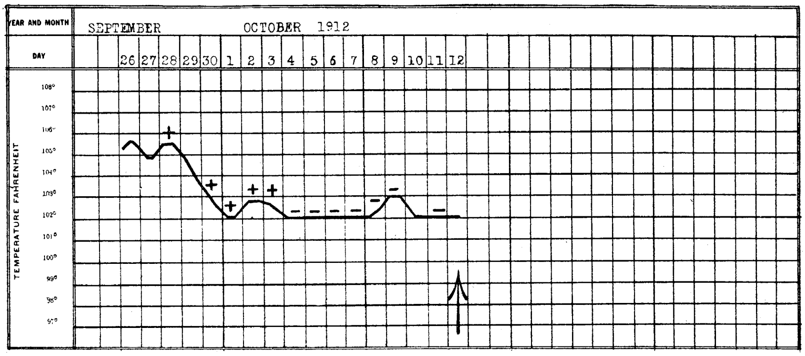
On September 26 two animals affected with hog cholera were brought to the laboratory. The blood of one of these hogs was examined immediately and the spirochetes were found with little difficulty. This animal was moribund, and was bled and examined on the same day. The other hog, No. 448, ultimately fully recovered from the disease. An examination of the temperature chart of Hog 448 will show the logical results obtained from the study of his blood on the dark field.

Three hogs, Nos. 450, 451, and 452, were inoculated with the Michigan (Demerick) strain of virus. The results of the dark field blood examinations appear in the charts below and were clearly confirmatory of previous findings.

Michigan (Rochester I) strain of virus.—On November 9, eight apparently normal pigs were received from Rochester, Mich. No particular notice was taken of these animals as the blood of other hogs suffering from cholera was at the time under close observation. The eight susceptible pigs were received in an isolated pen, at some distance from the experimental stable, and cared for by an attendant who never entered the experimental stable. This was the routine procedure when normal pigs were received.

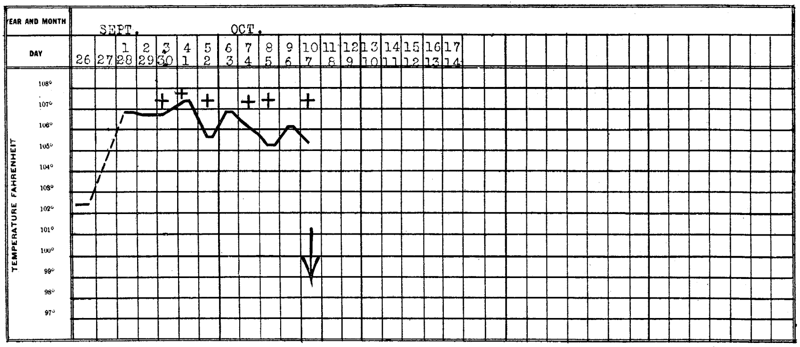
On the evening of November 12, Hog 503, one of the eight apparently normal pigs from Rochester, was removed to the experimental stable, inoculated with Michigan (Demerick) strain of virus and placed alone in a disinfected room. In this instance no dark field blood examination was made before inoculation, owing to the lack of time. It was planned that the examination of the blood, for the purpose of control, should be made the next morning following the inoculation. Likewise no temperature observation was made until the next morning. On November 13, the day following the inoculation of Hog 503, the dark field examination of the blood revealed the spirochete.

HOG NO. 448. MICHIGAN (DEMERICK) STRAIN OF VIRUS.



REMARKS: October 12. Animal fully recovered. Released as immune.

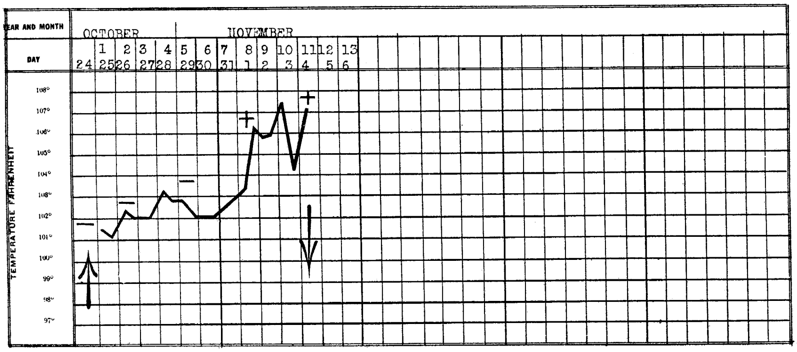
HOG NO. 450. MICHIGAN (DEMERICK) STRAIN OF VIRUS.



REMARKS: September 27. Animal inoculated with 4 c.c. Demerick virus.
October 5. Symptoms appeared.
October 14. Animal found dead and examined.

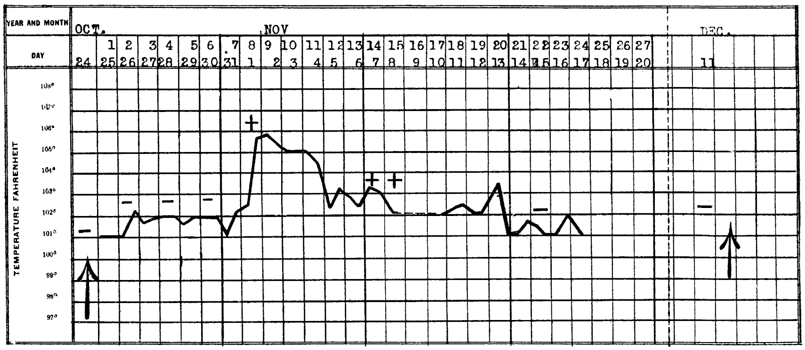
Large areas of congestion and hepatization in lower lobes of both lungs, kidneys contain a few petechiae. Spleen enlarged and gorged with blood. Mucosa of large intestine congested. Lymph glands enlarged and hemorrhagic.

HOG NO. 451. MICHIGAN (DEMERICK) STRAIN OF VIRUS.



REMARKS: October 24. Demerick virus (filtered through Berkefeld).
November 1. Symptoms developed.
November 4. Animal found dead and examined.
Lesions in lymphatic glands, spleen, lungs, and cecum typical of cholera.

HOG NO. 452. MICHIGAN (DEMERICK) STRAIN OF VIRUS.



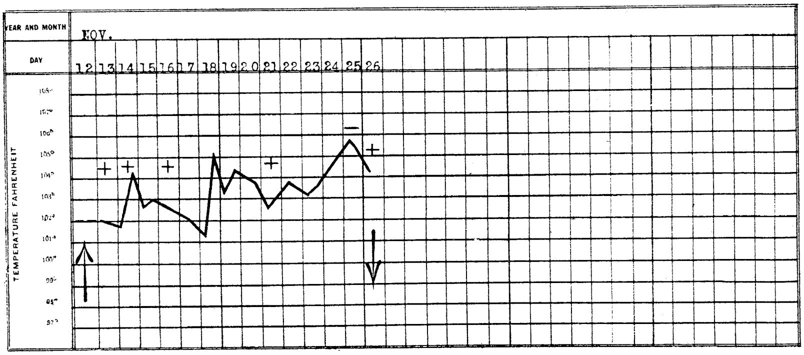
REMARKS: October 24. Inoculated with 5 c.c. of Michigan (Demerick) virus (unfiltered).
November 1. Symptoms appeared.
November 17. Animal recovered.
December 15. Animal released, immune.

The organism was again found on November 14. At this time the temperature of the animal was 104.2° F. and it showed symptoms of hog cholera. An inspection of the remaining hogs of this lot, which were still in the isolated pen used for receiving susceptible animals, showed that practically all were suffering from the disease. Among the apparent symptoms were diarrhea, anorexia, and listlessness.

Careful inquiry was made as to the source of this lot of pigs and it was found that they were purchased from a stock buyer, who had kept them for 10 days previous to receipt, in an inclosure which received all hogs purchased. Thus, it was clear that these pigs had been subjected to one of the most common methods of exposure to the disease, and that an incubation period of 10 days had elapsed before they were delivered at the laboratory.

In this instance a diagnosis of hog cholera was practically made by means of the dark field.

HOG 503. MICHIGAN (ROCHESTER) STRAIN OF VIRUS.



REMARKS:	November 12.	Inoculated with 4 c.c. virus from Hog 450 Michigan (Demerick) strain.
	November 14.	Found pig showing symptoms. One of Rochester hogs.
	November 17.	Typical symptoms.
	November 20.	Typical symptoms.
	November 26.	Animal moribund, bled and examined. Lymphatic glands, lungs, kidneys, and heart muscle show typical lesions.

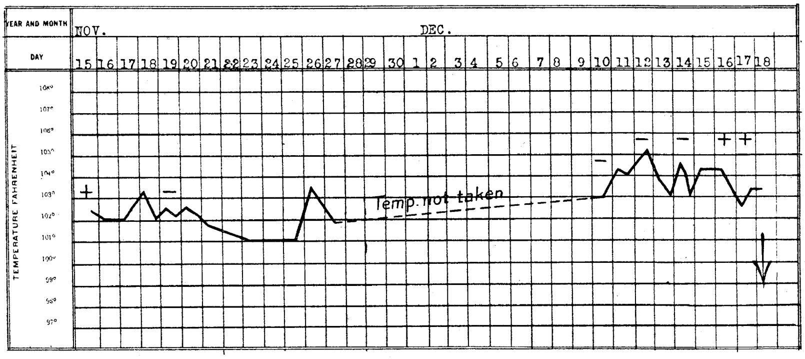
The autopsy findings in Hog 503, which was bled two weeks after being taken under observation, could not consistently be used to verify the diagnosis of cholera in this lot of hogs, because of the fact that this animal had received an inoculation of Michigan (Demerick) strain of virus.

Hog 504 was killed and examined on November 16, after two positive dark field examinations were made. The lesions were not pronounced because of the fact that sufficient time had not elapsed for typical lesions of cholera to develop.

The disease in this lot was of the subacute or chronic type as is shown by a study of the following charts of Hogs 505, 509, 510, 511, and 513. The clinical conditions and autopsy findings left no room for doubt as to the nature of the disease.

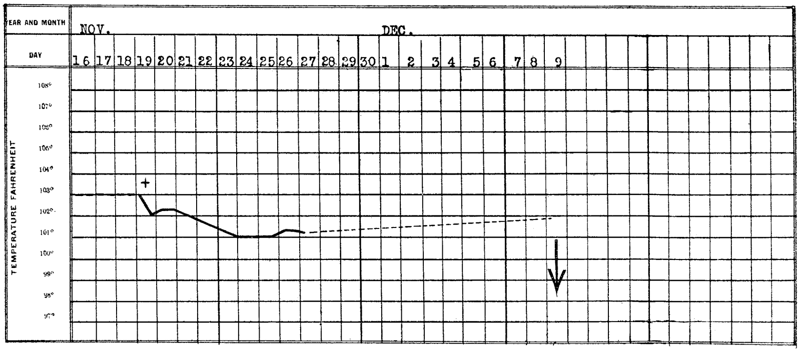
In further confirmation of the diagnosis of hog cholera in the above animals, when received at the laboratory, attention should be called to the chart of Hog 514. This animal remained in the isolated pen for susceptible hogs at the time the Rochester pigs

HOG NO. 505. MICHIGAN (ROCHESTER) STRAIN OF VIRUS.



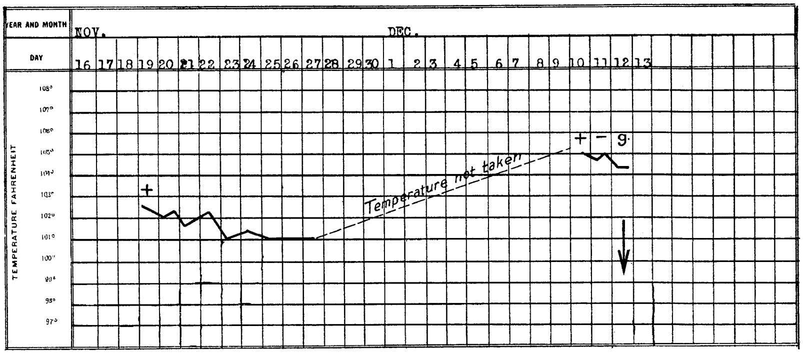
REMARKS: November 15. One of Rochester pigs. Not inoculated.
November 20. Symptoms of chronic type.
December 10. Animal very weak and emaciated.
December 19. Hog died on bleeding table.
Lymphatic glands, heart muscle, lungs, kidneys, and cecum show typical lesions of cholera.

HOG NO. 509. MICHIGAN (ROCHESTER) STRAIN OF VIRUS.



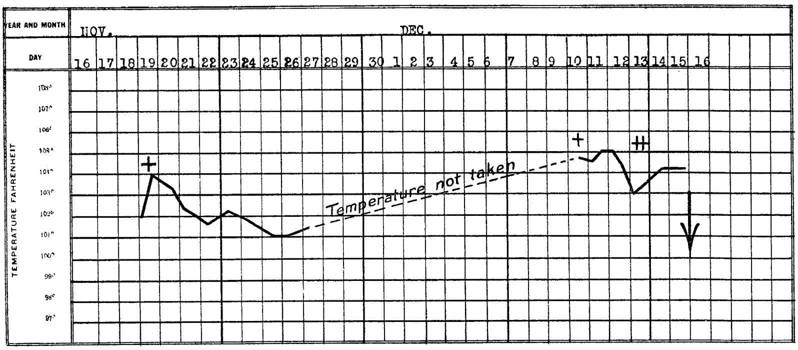
REMARKS: November 16. Rochester pig. Not inoculated.
November 20. Symptoms of chronic type.
December 9. Animal found dead.
Lymphatic glands, lungs, spleen, heart muscle, and kidneys show typical lesions.

HOG NO. 510. MICHIGAN (ROCHESTER) STRAIN OF VIRUS.



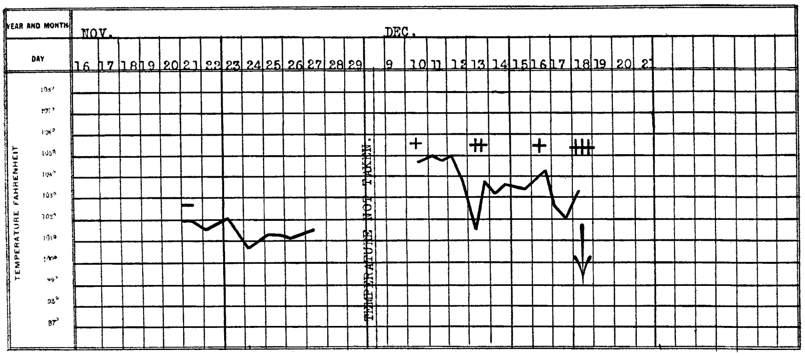
REMARKS: November 16. Rochester pig. Not inoculated.
November 20. Symptoms of subacute type.
December 10. Animal very weak and emaciated.
December 12. Animal moribund, bled and examined.
Spleen, lymphatic glands, and cecum show typical lesions of cholera.

HOG NO. 511. MICHIGAN (ROCHESTER) STRAIN OF VIRUS.



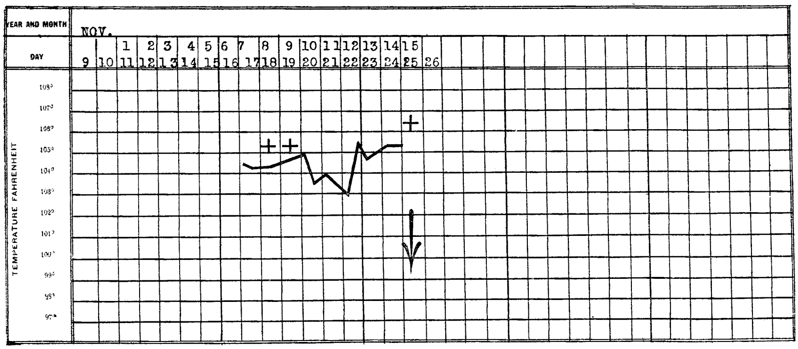
REMARKS: November 16. Rochester pig. Not inoculated.
November 20. No marked symptoms, looks fairly good.
December 1. Symptoms of subacute type.
December 10. Animal very weak and emaciated.
December 16. Found dead.
Typical lesions, lymphatic glands, kidneys, lungs, and cecum.

HOG NO. 513. MICHIGAN (ROCHESTER) STRAIN OF VIRUS.



REMARKS: Rochester pig, not inoculated.
November 20. No marked symptoms, but not doing well.
December 5. Subacute or chronic case.
December 10. Animal weak and emaciated.
December 18. Animal moribund, bled and examined.
Lungs, heart muscle, lymphatic glands, kidneys, and cecum show typical lesions.

HOG NO. 514. MICHIGAN (ROCHESTER) STRAIN OF VIRUS.



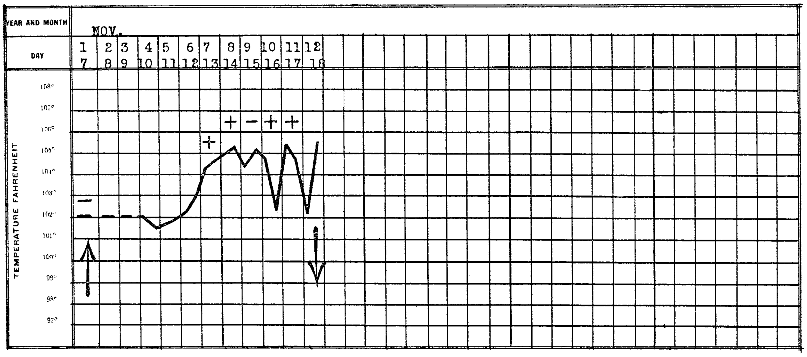
REMARKS: November 9–11. Rochester pigs were placed with this hog, which was normal.
November 25. Animal moribund, bled and examined.
Typical lesions, lymphatic glands, kidneys, lungs, and spleen.

were received. Hog 514 had been kept for a period of several weeks in the pen for "susceptibles" previous to this time and was in a healthy condition. The animal was not otherwise exposed to the disease, but promptly developed cholera about six days after the Rochester pigs were placed with it. The blood of Hog 514 showed the presence of the spirochete on three different examinations, and autopsy revealed typical lesions of the disease.

Indiana strain of virus.—This virus was secured from Mr. Delplane and was originally supplied by Dr. R. A. Craig, who reported that this strain was secured by him in the field some time ago and did not represent the B.A.I. strain of virus.

Hog 501 was inoculated with 5 c.c. of Indiana I virus on November 7, after having made a dark field examination of the normal blood. On the seventh day after inoculation the spirochete was found in the blood of this animal, although symptoms, with the exception of a beginning rise of temperature, were not observed until the following day. Attention should be called to the fact that on the ninth day after inoculation no spirochetes could be found in the blood, but characteristic granules were present, while on the tenth day the spirochete was again found. This phenomenon, observed in other cases as well, suggests that the spirochete under observation represents one stage in the life cycle of the organisms, and that the granule formation is possibly an important factor in its development.

Hog No. 501. Indiana I Strain of Virus.



Remarks:	November 7.	Inoculated with 5 c.c. of Indiana I virus.
	November 12.	4 P.M. Animal showed symptoms.
	November 18.	Animal moribund, bled and examined. Purple areas of discoloration on abdomen, nose, and ears. Subcutaneous blood extravasations. Lymphatic glands enlarged and hemorrhagic. Both kidneys petechiated and contain retention cysts. Hemorrhagic areas in heart muscle, lungs partially consolidated in both upper and lower lobes. Ulcers in cecum.

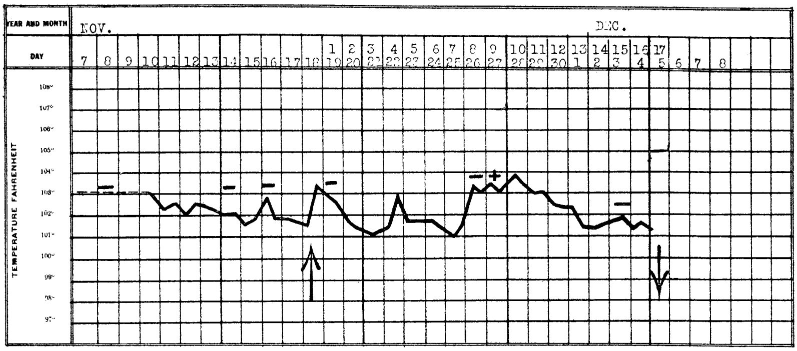
California (Hall) strain of virus.—Dr. Ivan C. Hall of California supplied this virus and the following history:

"We have just bled a hog originally injected with a virus obtained from one of our California towns. This virus was injected after being filtered and proven bacteria free, as it originally contained a pure culture of *B. cholera suis*.

"The hog became very sick about 10 days after and, appearing moribund, we bled him to death. The virus which I am sending you is some of this, after it has been diluted to one part in four and filtered through a Berkefeld filter."

Hog 502, which received the above strain of virus, had been kept for several days as a control on the isolation of the experiments and on the dark field examinations of the blood of normal and diseased hogs. Nine days after inoculation this animal exhibited mild symptoms of hog cholera, with temperature of 103.5° F. The spirochete was found in its blood. Fifteen days after inoculation its blood was free from spirochetes and characteristic granules. On the 17th day it was killed by large male Hog 400, and at autopsy no pathological lesions could be found.

HOG NO. 502. CALIFORNIA (HALL) STRAIN OF VIRUS.



REMARKS: November 18. Inoculated with 15 c.c. California (Hall) virus (diluted 1–4) and filtered through Berkefeld.
November 26–30. Slight symptoms.
December 3. Animal apparently normal.
December 5. Animal killed by large male Hog No. 400. No lesions.

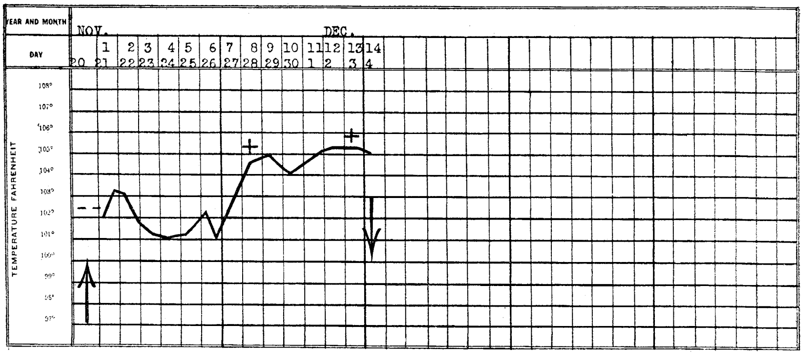
Ohio (*Pettigrew*, *Hazen*, *and Heinz*) *strains of virus*.—These viruses, obtained through the kindness of Dr. Paul Fischer and Dr. A. D. Fitzgerald, were from three different outbreaks and represented mixtures of blood samples from the number of pigs indicated on the labels. Ohio (Pettigrew), Ohio (Hazen), and Ohio (Heinz) represented mixtures of virulent blood from three, five, and six pigs respectively.

A dark field examination was made of the undiluted Ohio (Pettigrew) virus, as found in the original container, and one spirochete was observed.

The spirochetes were found in the blood of hogs inoculated with all three strains of Ohio virus, as shown on the following charts. The blood of Hog 521 (Ohio, Heinz strain) on the fifth day after inoculation contained relatively numerous spirochetes. On the seventh day none could be found, while on the eighth day the organism was readily found. By the eleventh day the clinical condition was much improved and the findings were negative, but the animal suffered a relapse and on the day of death the organisms were again present in the blood.

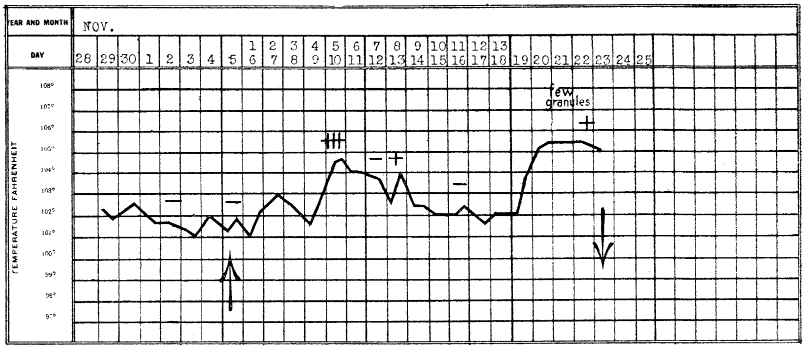
Hog 525, inoculated with the Ohio (Hazen) strain of virus, manifested symptoms on the fifth day after inoculation, and on that day the findings were positive. This animal is now fully recovered and no spirochetes are present in his blood.

HOG NO. 515. OHIO (PETTIGREW) STRAIN OF VIRUS.



REMARKS: November 20. Inoculated with 15 c.c. filtered (Pettigrew) Ohio virus, diluted with normal saline 1–4.
November 29. Animal quite sick.
December 4. Animal moribund, bled and examined.
Spleen, kidneys, lymphatic glands, liver, lungs, and cecum show typical lesions.

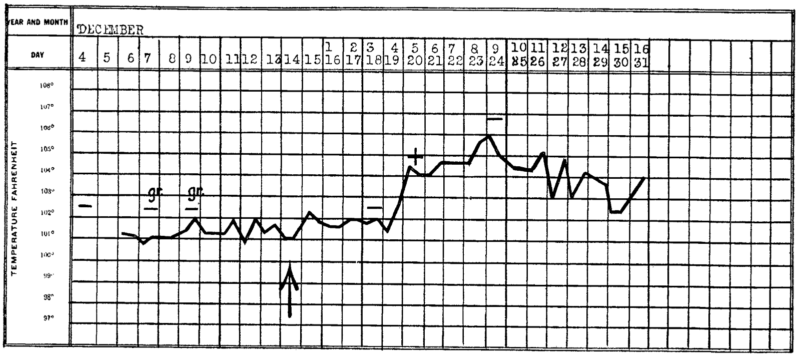
HOG NO. 521. OHIO (HEINZ) STRAIN OF VIRUS.



REMARKS: December 2. Hog in good condition.
December 5. Inoculated with 15 c.c. Ohio (Heinz) virus, Berkefeld filtered and diluted 1–4.
December 10. Symptoms appeared.
December 15. Condition of animal improving.
December 19. Animal suffered relapse.
December 23. Animal died.
Ventral surface of body deep purple color. Extravasations in subcutaneous tissue. Inguinal glands large and hemorrhagic. Lungs, both involved in all portions, highly congested, partially consolidated in small areas. Bronchial tubes filled with exudate. Heart, petechiae in auricles and the heart muscle. Both kidneys show small petechiae. Liver, passive congestion. Mesenteric and retroperitoneal and inguinal lymph glands enlarged and hemorrhagic. Cecum shows ulcers.

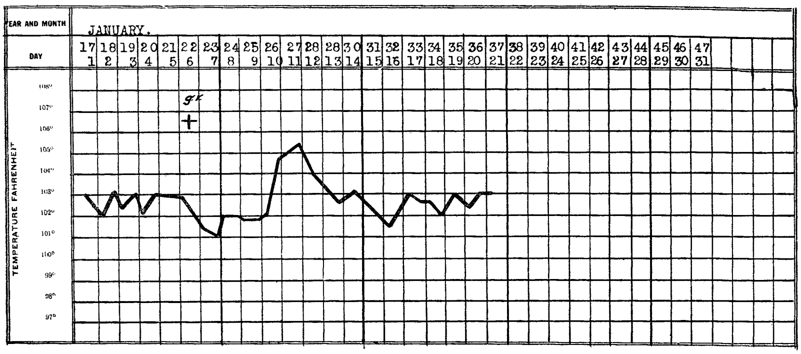
Kansas I strain of virus.—Professor L. D. Bushnell supplied this virus and described it as follows: "I sent you a couple of ounces of hog cholera blood, which was collected from an outbreak about a mile east of Manhattan, Kan., by Doctor Gingery. This animal was killed and showed typical lesions. It had a temperature of 108°. Twelve animals had died when the herd was vaccinated."

HOG NO. 525. OHIO (HAZEN) STRAIN OF VIRUS.



REMARKS: December 4. Susceptible pig; fine condition.
December 14. Inoculated 15 c.c. Ohio (Hazen) virus diluted 1-4 and Berkefeld filtered.
December 20. Anorexia, listless.

HOG NO. 525—*Continued.*

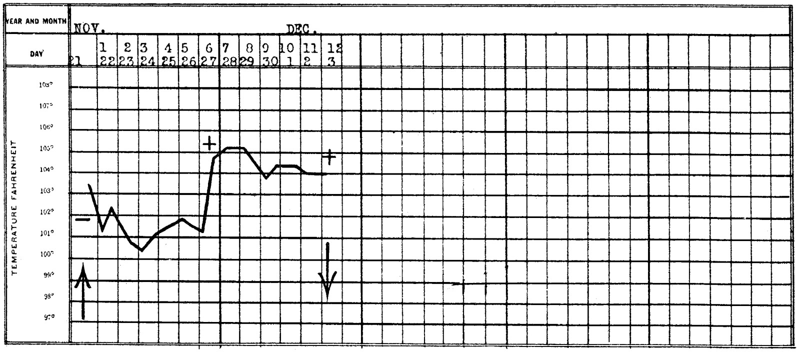


REMARKS: January 21. Animal fully recovered, released.

This virus, when received, was examined on the dark field. It was found to contain numerous granules, some bacteria, but no spirochetes were observed. The blood of Hog 516 inoculated with this virus showed the presence of the spirochete.

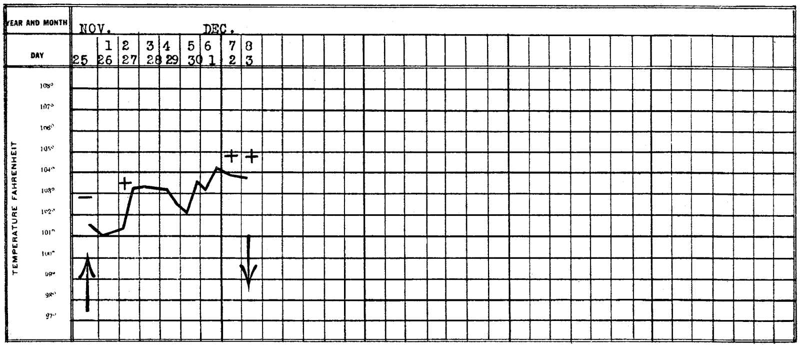
Unknown strain of virus.—On November 25 and 26, Hogs 517 and 518 were inoculated with California (Hall) and California (University) strains of virus respectively. Before inoculation, blood from these apparently normal animals was collected in sterile sodium citrate solution for control examination. In the case of Hog 518 the dark field examination of the assumed normal blood revealed the presence of the spirochete. Subsequent developments clearly indicated that these animals were in some way exposed to the disease before inoculation, as clinical evidences of the disease were clearly present in both cases within a day or two following inoculation, an interval of too short duration to correspond with the incubation period which should follow the injection of virus. Moreover Hogs 517 and 518 were both in a moribund condition,

HOG NO. 516. KANSAS I STRAIN OF VIRUS.



REMARKS: November 21. Inoculated with 4 c.c. Berkefeld filtered Kansas I virus.
November 29. Animal very sick.
December 3. Animal moribund, bled and examined.
Typical lesions, marked in lymphatic glands, kidneys, cecum, spleen, and lungs.

HOG NO. 517. UNKNOWN STRAIN OF VIRUS.



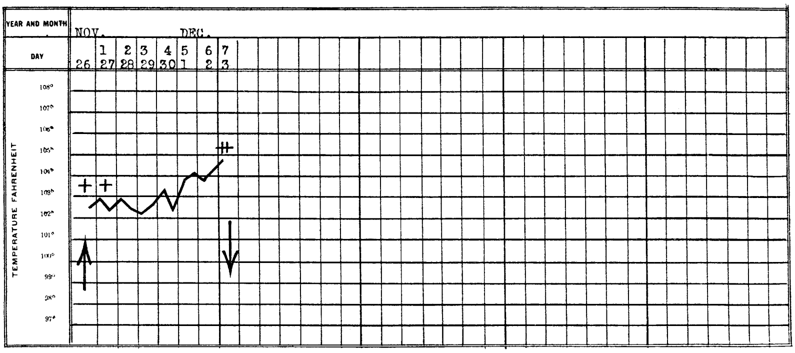
REMARKS: November 25. Inoculated with 15 c.c. California (Hall) virus, Berkefeld filtered and diluted 1-4.
November 26. Animal shows symptoms, evidently due to previous natural exposure.
December 3. Animal moribund, bled and examined.
Cecum, lymphatic glands, spleen, kidneys, and lungs show typical lesions.

eight and seven days respectively, following the inoculation, periods relatively shorter than the average duration of the course of the disease of cases included in this work.

California (*University*) *strain of virus.*—Dr. C. M. Haring and Dr. F. J. Mitchell kindly furnished this specimen of virus. The history of the material follows: "This particular virus I am sending comes from an outbreak of a not severe type, but the virus has been killing pigs now in from seven to twelve days for the last four months. Pigs showed petechiae in the lungs and kidneys, very few on the intestines. Ulcers are sometimes present, sometimes not."

This strain of virus was injected into Hog 524 after having established three negative dark field examinations of his blood. As shown on the chart the spirochetes were observed at three different times during the course of the disease. Again, in this case, the spirochete was found about 24 hours before any clinical symptoms or rise of temperature occurred.

HOG NO. 518. UNKNOWN STRAIN OF VIRUS.



REMARKS: Inoculated November 26, with 15 c.c. University of California virus, filtered and diluted 1-4.

November 28. Not eating well, red spots on ventral surface of body. Probably infected with natural exposure previous to inoculation.

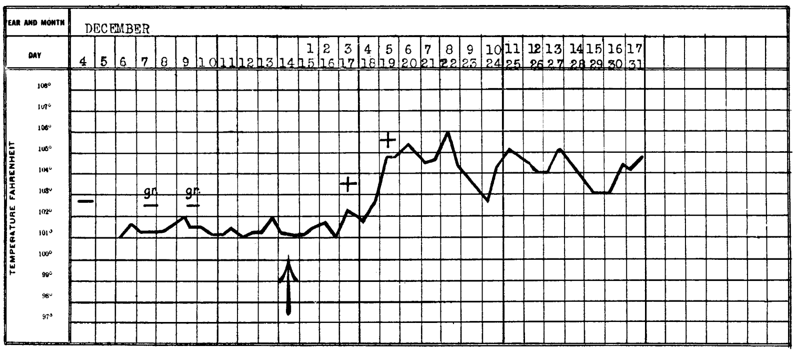
December 3. Animal moribund, bled and examined.

Small blood extravasations in muscular and subcutaneous tissue. Spleen slightly enlarged, dark gorged with blood. Capsules of both kidneys loosely adherent, and both kidneys filled with small urinary cysts. Both lungs show areas of consolidation. Inguinal and mesentery glands enlarged and hemorrhagic. Ulcers present in cecum.

Michigan (*Rochester II*) *strain of virus.*—On January 10, 1913, 15 apparently normal hogs were received from Rochester, Mich. These pigs, averaging about 40 pounds in weight, while not in thrifty condition, did not appear to be diseased.

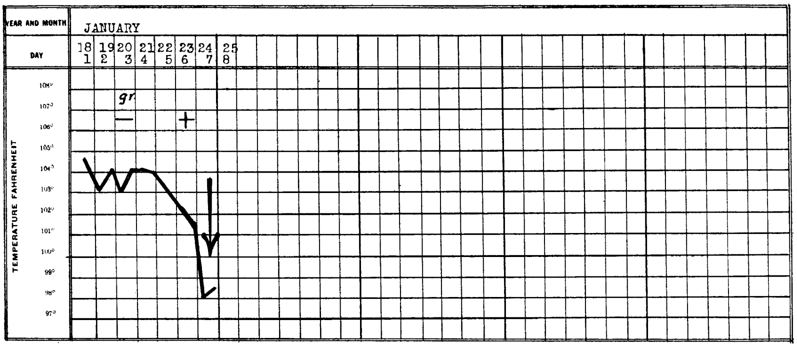
On January 11, dark field examinations of the blood of two of these animals revealed the presence of the spirochetes. The temperatures of these two animals were each 103.8° F. and a tentative diagnosis of the chronic type of cholera was made. On January 13, three days after the receipt at the laboratory, the temperatures of seven of the 15 animals ranged between 104° and 105.5° F. and, from other symptoms, there was no doubt but that the diagnosis of cholera was correct. Investigation showed that on January 1, these animals were placed under such conditions that exposure to hog cholera might result. As they were received at the laboratory on January 10 and symptoms definitely established by January 13, there existed a period of incubation corresponding to that usually present in the chronic or subacute types of cholera.

HOG NO. 524. CALIFORNIA (UNIVERSITY) STRAIN OF VIRUS.



REMARKS: December 4. Susceptible pig; fine condition.
December 14. Inoculated 15 c.c. California (University) virus, Berkefeld filtered and diluted 1–4.
December 18. "Off feed." Symptoms appeared.

HOG NO. 524—*Continued.*



REMARKS: January 8. Found dead.
Typical lesions, lungs, liver, kidney, spleen, cecum, inguinal glands, large intestine, and subcutaneous tissue.

These animals are under observation at the present time. A summary of the dark field findings is presented in the following table.

TABLE 1.

MICHIGAN (ROCHESTER II) STRAIN OF VIRUS.

Hog	Number Dark Field Examinations to Date	Presence of Spirochete in Blood	Remarks
537	3	+++	Temp. range from 102 –104.8° F.
538	3	+++	“ “ “ 101.2–104.1 “
539	3	++–	“ “ “ 102.2–105.5 “
540	1	–	“ “ “ 101.6–103.5 “
541	1	+	“ “ “ 101.6–103.2 “
542	3	++–	“ “ “ 102. –104.6 “
543	1	+	“ “ “ 102. –103.5 “
544	2	++	“ “ “ 102.4–105.5 “
545	1	–	“ “ “ 101.8–103. “
546	1	–	“ “ “ 101. –104.5 “
547	2	––	“ “ “ 101.5–103. “
548	2	++	“ “ “ 102. –105. “
550	1	–	“ “ “ 101.5–103. “
551	1	–	“ “ “ 102. –103.5 “
552	1	+	“ “ “ 101.6–103.8 “

DARK FIELD EXAMINATIONS OF THE BLOOD OF NORMAL HOGS.

In addition to the control examinations which were made in practically all cases, as shown on the charts above, additional data relative to negative findings in normal blood have been secured.

TABLE 2.

DARK FIELD EXAMINATIONS OF THE BLOOD OF HOGS NOT SUFFERING FROM HOG CHOLERA.

Hog	Number Dark Field Examinations	Spirochete Present or Absent	Condition of Animal
522	2	––	Normal
512	1	–	“
523	1	–	“
526	2	––	“
519	2	––	“
520	2	––	“
527	3	–––	“
528	1	–	Infested with Ascaroides
529	2	––	“ “ “
530	1	–	“ “ “
531	1	–	“ “ “
532	1	–	“ “ “
533	1	–	“ “ “
534	1	–	“ “ “
535	1	–	“ “ “
536	1	–	“ “ “
400	1	–	Natural immune
553	2	––	Normal
554	2	––	“
555	2	––	“
556	2	––	“

A number of hogs, planned for material to be used in the work, became badly infested with intestinal parasites (chiefly *Ascaroides suella*) and were not utilized except for control blood examinations. Some of these animals exhibited temperatures as high as 106° F.

at the time of the dark field examinations. The negative findings in these cases, therefore, present valuable data in that they show that the presence of the spirochete observed in the blood of animals suffering from cholera, does not depend merely upon a febrile condition of the blood.

Several immune hogs not included in the above data also served as donors of normal blood specimens. These additional negative findings in the blood of hogs not infected with hog cholera are shown in Table 2.

HORSE SERUM VIRUS PHENOMENON.

Horse serum virus may be produced by securing blood from a horse one to two hours after the animal has received an intravenous injection of approximately 150 c.c. of virulent hog cholera serum. The character of this material and the results of experiments, which demonstrate that it is not a mere dilution of hog cholera serum, have been reported in former publications.[1] It may be concisely stated that hog cholera virus undergoes some inexplainable process of activation after approximately one hour's residence in the circulatory system of the horse.

On October 25 the blood of Horse 1 was examined on the dark field. Nothing other than erythrocytes, leukocytes, and some blood dust was noted. At 1:45 P.M. on October 25, Horse 1 was given an intravenous injection (jugular vein) of 150 c.c. B.A.I. strain of virus. A specimen of this hog cholera serum was examined on the dark field and no spirochetes were found, although the material did contain many characteristic granules. The animal was led from the stocks and immediately became groggy and weak, the pulse was weak, respiration labored, eye dull and listless, and peristalsis violently active. The animal became covered with a profuse cold perspiration and frequent watery evacuations followed. Three-fourths of an hour after the injection of the horse it was bled under aseptic conditions and the blood examined by dark field illumination. The spirochetes were found with ease, six organisms being found in a few minutes. Blood drawn from the horse one and three-fourths hours after injection also contained the spirochete when examined on the dark field. The one and three-fourths hours virus also contained a marked increase in the number of granules. A specimen of the blood of Horse 1 drawn two and three-fourths hours after injection upon careful dark field examination revealed only granules and refractive bodies—no spirochetes could be found.

The virulence of the three-fourths-hour horse serum virus which the above animal yielded was tested by the inoculation of Hog 4501. The result of this animal inoculation conformed with extensive data previously reported, in that the hog became the subject of a more acute type of cholera after a relatively short period of incubation, as compared with the average case following the injection of hog cholera serum itself.

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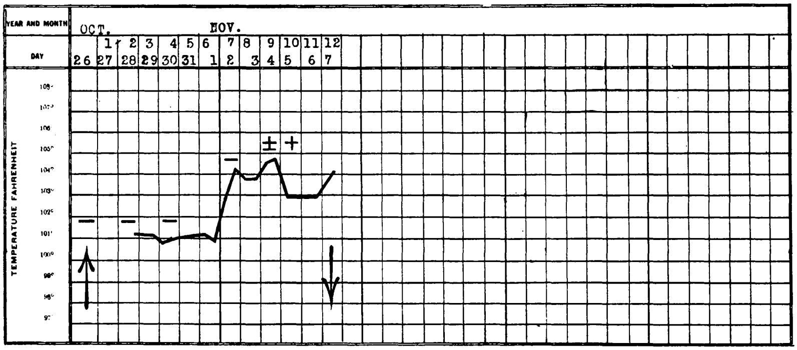
[1] King and Wilson, *Bull. Kansas Exp. Sta.*, 1910, No. 171. *Jour. Infect. Dis.*, 1912, 11, p. 441; *Ztschr. f. Immunitätsf. u. Exp. Ther.*, 1913, 16, p. 367.

On November 22, Horse 2 was utilized in duplicating the above experiment, after establishing a normal condition of the blood in so far as absence of spirochetes was concerned. This animal was injected intravenously with 180 c.c. of B.A.I. and Indiana I strains of virus mixed. The symptoms were of the usual character, but were more retarded and less pronounced than those recorded in the first experiment with Horse 1. In our experience,[1] more pronounced reactions following injection and more uniform horse serum virus result, when the animals have been subjected to one or more repeated injections with the hog cholera virus, a condition in all probability not depending upon anaphylaxis as shown by previous results. Horse 1, above, had been repeatedly used on the work; Horse 2 had never been subjected to the treatment before.

HOG NO. 4501. B.A.I. STRAIN OF VIRUS. HORSE SERUM VIRUS.



REMARKS: October 26. Inoculated with 4 c.c. activated horse serum virus.
November 2. Symptoms appeared.
November 7. Animal found dead and examined.
Many subcutaneous blood extravasations. Muscle of right ventricle contains blood extravasations. Lower lobe left lung consolidated. Areas of red hepatization in upper lobes of both lungs. Spleen soft, dark, and gorged with blood. Kidneys show numerous petechiae. Large ulcer near ileo cecal valve. Extensive areas of congestion in intestinal mucosa. Lymphatic glands enlarged and hemorrhagic.

The three-fourths-hour horse serum virus of Horse 2 showed the spirochetes on the dark field. In the two-hour virus only one spirochete could be found.

The above data must be verified by repeated experiments. However, assuming that the spirochete under observation, in different stages of its life cycle, might represent the etiological factor in hog cholera, it would not be impossible to explain the horse serum virus phenomenon by the results obtained above.

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[1] Grateful acknowledgment is made to Dr. R. H. Wilson, who for several years has been associated with the senior author in this work, and who rendered valuable assistance in carrying out the horse serum virus experiments included in the present investigation.

SUMMARY.

The results may be summarized as follows:

1. Dark field examinations of the blood of 48 hogs, all normal, excepting some which were infested with intestinal parasites, have revealed no spirochetes. All of these specimens have been relatively free from granules, with the exception of some instances, when it was usually possible to determine that the granules were liberated from crushed leukocytes.

2. The examination of the blood of all hogs—40 animals—which were suffering from hog cholera, revealed the presence of a spirochete. All specimens of blood from these animals, during the height of the disease, contained characteristic granules.

3. Negative dark field findings followed positive findings in the case of six hogs which recovered from the disease.

4. The blood of two naturally immune hogs was free from spirochetes and granules.

5. The spirochetes and granules have been observed in hogs infected with 12 different strains of virus, as follows: B.A.I., Michigan (Demerick), Michigan (Rochester I), Indiana I, California (Hall), Ohio (Pettigrew), Ohio (Hazen), Ohio (Heinz), Kansas I, Unknown, California (University), and Michigan (Rochester II).

6. Hog cholera was diagnosed in two small herds of hogs after receipt of the animals at the laboratory, by means of the dark field examination of the blood. In other instances, positive dark field findings were secured a few hours before any symptoms appeared.

7. The spirochetes were found with less difficulty in the blood of hogs suffering from the acute form of the disease.

8. In two experiments the spirochetes were demonstrated in horse serum virus.

9. Spirochetes are uniformly demonstrable in the intestinal ulcers of hogs dead from cholera.

CONCLUSIONS.

Without cultures with which to inoculate susceptible animals it is, of course, impossible at this time to definitely ascribe to the spirochete under observation any pathogenic properties or etiological significance. Nevertheless, careful consideration should be given to the results which are consistent and uniform throughout, and which, in some particulars, strongly suggest the relationship of this spirochete to hog cholera, as a causative factor. As this organism has not been previously reported and described the name *Spirochaeta suis* may properly be suggested.